Simulating the impact of climate change (elevated CO_2 and temperature, and water deficit) on the growth of red and white Tempranillo grapevine in three consecutive growing seasons (2013–2015)

T. Kizildeniz[a], J.J Irigoyen[a], I. Pascual[a], F. Morales[b,c,*]

[a] Universidad de Navarra, Dpto. de Biología Ambiental, Grupo de Fisiología del Estrés en Plantas, Unidad Asociada al CSIC (EEAD, Zaragoza e ICVV, Logroño), Facultad de Ciencias, Irunlarrea 1, 31008, Pamplona, Spain
[b] Estación Experimental de Aula Dei (EEAD), CSIC, Dpto. Nutrición Vegetal, Apdo. 13034, 50080, Zaragoza, Spain
[c] Instituto de Agrobiotecnología (IdAB), Universidad Pública de Navarra-CSIC-Gobierno de Navarra, Campus de Arrosadía, 31192, Mutilva Baja, Spain

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ABSTRACT

In recent decades, agricultural production is being affected by a sharp increase in atmospheric CO_2 concentration. Due to the greenhouse effect gases, crops are impacted by enhanced temperatures and concomitantly by increased scarce water availability. All arid and semiarid areas, including Mediterranean viticulture, must face these three climate change-related factors: atmospheric CO_2 concentration and temperature increases, and scarce water for irrigation. Scarce water is a problem even in irrigated viticulture, as irrigation is becoming more and more restricted. Within this context, the aim of this work was to investigate grapevine (Vitis vinifera L. cv. red and white Tempranillo) vegetative and reproductive growth. Fruit-bearing cuttings were grown under elevated CO_2 (around 700 $\mu mol\ mol^{-1}$ or ppm, versus 400), high temperature (ambient temperature + 4 °C, versus ambient) and water deficit (cyclic drought, versus full irrigated) in temperature gradient greenhouses for three consecutive growing seasons (years 2013, 2014 and 2015). Climate change impacted markedly vegetative growth. Within the abovementioned factors, vegetative growth (total vegetative mass) was significantly reduced by drought (consistent the three years) and was associated to a low substrate water status and low leaf stomatal conductance. Elevated CO_2 stimulated total vegetative mass, whereas leaf area was not affected. When plants were grown under elevated CO_2, the largest increases were observed in leaf (white) and root (red) growth. In these experiments, elevated CO_2 did not compensate the negative effects of water stress. An increase of the mean temperature 4 °C had no consequences on vegetative growth. Yield and yield-related traits were unaffected by the climate change scenario.

1. Introduction

Greenhouse effect gases, such as CO_2, chlorine-fluorine-carbon (CFC) and its derivatives, CH_4 and N_2O, are causing the recent increases in temperature at a global scale. Atmospheric CO_2 concentration has increased since preindustrial period from 280 $\mu mol\ mol^{-1}$ (ppm) to currently more than 400, and the predictions for the end of the century range from 421 to 936 (Meinshausen et al., 2011). Cumulative emissions of CO_2 contribute largely to the global mean surface warming. Foreseen values range from stabilization at 1.5 °C higher than the current reference period (1986–2005) to a more than 4 °C increase, depending on the mitigation measures adopted (IPCC, 2014). During the last two decades, for instance, air temperature has increased 0.85 °C

(IPCC, 2014). This greenhouse effect is impacting crops, causing more frequent Mediterranean crop drought (NOAA, 2011) that results from reduced water supply and increased transpiration due to the higher temperature. Grapevine is a typical Mediterranean crop fully adapted to summer drought, but its response to climate change-related factors (elevated CO_2, high temperature and drought) can vary when stress factors act individually and/or interacting (Kizildeniz et al., 2015).

An instantaneous response to an elevated atmospheric CO_2 is an increased photosynthesis and a decreased transpiration, due to higher substrate availability for C fixation and stomata partial closure respectively (Lambers et al., 1998; Long, 1991). Consequently, elevated CO_2 was reported to stimulate plant growth in different species (Ainsworth and Long, 2005; Drake et al., 1997; Kimball and Idso,

1983). As in other C_3 species where photosynthesis is limited by CO_2 availability, grapevine responds to the elevated CO_2 with an increased vegetative growth and yield (Bindi et al., 1996; Bowes, 1993; Rogers et al., 1994). However, long-term CO_2 exposure leads to a phenomenon known as photosynthetic acclimation or down regulation that decreases grapevine photosynthetic capacity (Salazar-Parra et al., 2015), resulting in less clear effects on grapevine physiology and growth. Secondary changes in morphology, allocation and chemical composition may also influence growth (Poorter et al., 1997).

Studies of elevated temperature on grapevine have been focused on berry composition, due to its association with grape ripening (Kuhn et al., 2014). Besides its effects on berries, depending on the level of elevated temperature and duration, it could also affect grapevine physiology, in particular photosynthesis, and vigor. Rates of photosynthesis can be reduced 30–50% when temperature rises over the 30 °C light-saturated optimum (Greer and Weedon, 2013). Photosynthesis decreases with increasing temperatures were attributed only 15–30% to stomatal closure (Greer and Weedon, 2012; Zsófi et al., 2009). More extreme temperatures can also inactivate the CO_2 fixing Rubisco (Ribulose-1,5-biphosphate carboxylase oxygenase) enzyme, which results in a drastic reduction of photosynthesis and limits the berry sugar supply causing slow ripening (Greer and Weedon, 2013). As a consequence of photosynthesis (and sugar accumulation) decreases under high temperatures, growth and yield are expected to decrease (Medrano et al., 2003).

Grapevine is traditionally a non-irrigated crop that is cultivated on 6 out of 7 continents (Hofmann and Schultz, 2015), where it occupies quite extensive agricultural areas in arid and semiarid regions. Water is the most limiting resource in these environments, such as the Mediterranean region, where rainfall is scarce and irregularly distributed along the year. Climate change models predict even more arid conditions for the future (Ashour and Al-Najar, 2012; Collins et al., 2013). Grapevine has developed physiological and morphological mechanisms enabling it to produce under such circumstances (Koundouras et al., 2008). Irrigation is a major tool for grapevine growers to increase soil water availability, which should lead to potential higher yields with (Salon et al., 2005; Valdes et al., 2009; Williams and Matthews, 1990) or without (Reynolds et al., 2007; Smart and Coombe, 1983) quality losses. Depending on the intensity of water stress and on the grapevine developmental stage, soil water availability nevertheless has more influence on grapevine vegetative growth than on yield (Kizildeniz et al., 2015; Korkutal et al., 2011; Williams and Matthews, 1990). For viticulture purposes, a managed deficit irrigation may be used to control grapevine vegetative growth and quality (Kennedy et al., 2002).

In the literature, effects of climate change (elevated CO_2, high temperature and water scarcity) on grapevine physiology have been investigated as individual effects more than the interactions among them. When individual effects are considered, exposure time, duration and sequence are essential for growth. For instance, increased number of warm days during flowering-veraison period and low precipitation during maturation result in greater berry weights (Jones and Davis, 2000). Combination of all abovementioned climate change-related factors has more severe effects on berry weight (McCarthy, 1997). When high temperature and water deficit are combined, leaf water potential, stomatal conductance, and net photosynthesis are reduced (Edwards et al., 2011). In the long-term, if these factors persist, the leaf area can be affected. Stimulation of grapevine production by elevated CO_2 is decreased or completely cancelled in presence of high temperature (Bindi et al., 2001).

Grapevine works focused on multiple stress factors are rarely performed because such studies in the field or under controlled conditions are complex, difficult and expensive to execute. The aim of this study was to analyze the influence of the three main climate change-related factors (elevated CO_2, high temperature and water scarcity), acting both individually and/or interacting, on plant vegetative and reproductive (yield) growth in fruit-bearing cuttings of two grapevine cultivars (red and white Tempranillo). Plants were grown in temperature gradient greenhouses (TGGs) during 3 consecutive growing seasons (2013, 2014 and 2015).

2. Materials and methods

2.1. Plant material

Dormant cuttings of *Vitis vinifera* L. cv. red (accession T43, clone RJ-43) and white Tempranillo (accession CI-101 in "La Grajera" germplasm bank, Riojás Government, Spain) were sampled from an experimental vineyard of the Institute of Sciences of Vine and Wine (ICVV) in Logroño (La Rioja, Spain). Experiments were conducted using each year new cuttings (not imposing treatments on the same plants for 3 consecutive years). Tempranillo is a local red grape variety widely cultivated in northern and central Spain, currently expanded to many other viticulture areas in the world. A new white berry somatic variant of Tempranillo ("white Tempranillo") is the result of a spontaneous, natural mutation, which was discovered in an old red Tempranillo vineyard in 1988 in Murillo de Río Leza, La Rioja, Spain (García-Escudero et al., 2011) and vegetatively propagated from one single grapevine plant.

2.2. Growth conditions

We used two types of greenhouses for growing the plants. Until fruit set, plants were grown in a pre-culture greenhouse with an effective cooling system where temperature and relative humidity (RH) were set at 26/15 °C and 60/80% (day/night) respectively and with a photoperiod of 15 h using natural daylight supplemented with high-pressure metal halide lamps (OSRAM®, Augsburg, Germany), providing a photosynthetic photon flux density (PPFD) of 500 µmol m^{-2} s^{-1} at inflorescence level. Variability around target temperature and RH values was typically 1.5–2% and 8–12%, respectively (Morales et al., 2014). After fruit set, and coincident with the start of the treatments, plants were moved to the second greenhouses, i.e., the temperature-gradient greenhouses (TGGs). Table 1 summarizes temperature and RH data within the TGGs in 2013, 2014 and 2015.

Fruit-bearing cuttings were obtained according to Mullins (1966), as described by Kizildeniz et al. (2015). Briefly, cuttings were treated with indole butyric acid (300 mg L^{-1}) in a heated rockwool moist-bed (25–27 °C) kept in a cool chamber (5 °C). One month later, the rooted-cuttings were planted in 0.8 L plastic pots containing a mixture of sand, perlite and vermiculite (1:1:1, in volume) and transferred to the pre-culture greenhouse. Only a single flowering stem was allowed to develop on each plant, in order to get only one berry bunch per plant. Until fruit set, vegetation was controlled and only 4 leaves per plant was allowed to grow. At fruit-set, the cuttings were transplanted to 13 L plastic pots including a peat and perlite (2:1, v/v) mixture.

Field capacity, permanent wilting point and available water content of the peat:perlite mixture were determined using a soil moisture equipment with ceramic plates of 33 and 1500 kPa (Richards, 1941). Bulk density was calculated weighing the dry substrate contained in a given volume. The characteristics of the peat-perlite mixture used with

Table 1

Temperature and relative humidity recorded in the temperature gradient greenhouse experiments with red and white Tempranillo grapevine. T, ambient temperature and T + 4 °C, ambient temperature + 4 °C.

Year	2013		2014		2015	
Temperature regime	T	T + 4 °C	T	T + 4 °C	T	T + 4 °C
Daily mean temp. (°C)	21.2	24.2	22.1	26.5	22.9	27.1
Days above 35 °C	2	36	8	42	19	50
Daily mean RH (%)	64.9	57.0	65.4	52.1	63.7	52.7

Table 2

Substrate (peat and perlite (2:1, v/v) mixture) characterization with respect to dynamics of water content.

Field capacity (-33 kPa)	613 g H_2O L^{-1} substrate
Permanent wilting point (-1500 kPa)	106 g H_2O L^{-1} substrate
Available water content	507 g H_2O L^{-1} substrate
Bulk density	0.159 g cm^{-3}

respect to dynamics of water content are summarized in Table 2.

Nutrient solution was applied as proposed by Ollat et al. (1998): NH_4NO_3 (64.5 mg L^{-1}), $(NH_4)_2HPO_4$ (75 mg L^{-1}), KNO_3 (129 mg L^{-1}), $MgSO_4 \cdot 7H_2O$ (125 mg L^{-1}), $Ca(NO_3)_2 \cdot 4H_2O$ (248 mg L^{-1}), $(NH_4)_2SO_4$ (66 mg L^{-1}), Fe-EDDHA (280 mg L^{-1}), H_3BO_3 (2.86 mg L^{-1}), $MnCl_2 \cdot 4H_2O$ (1.81 mg L^{-1}), $ZnSO_4 \cdot 7H_2O$ (0.22 mg L^{-1}), $CuSO_4 \cdot 5H_2O$ (0.08 mg L^{-1}) and $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (0.016 mg L^{-1}). These growing conditions were maintained until fruit set from March to May 2013, 2014 and 2015.

2.3. Temperature gradient greenhouses and experimental design

Treatments were applied in the second type of greenhouses, the temperature gradient greenhouses (TGGs), located in the campus of the University of Navarra (42°48′N, 1°40′W; Pamplona, Navarra, Spain), from June to August 2013, 2014 and 2015 (fruit set to maturity). TGGs have a design based on temperature gradient tunnels (Rawson et al., 1995), which allows investigating the effects on plants of environmental changes, such as elevated temperature, elevated CO_2 and drought, acting separately or in combination. TGGs contain 3 temperature modules (3.04 m long each), CO_2 can be injected inside increasing the air CO_2 concentration as desired, and a gradient of temperature is created along them (from module 1 of ambient temperature to module 3 of ambient $+4$ °C) (Morales et al., 2014). Also, plants growing inside the TGGs can be subjected to different levels of irrigation. Thus, two TGGs were set at current (400 ppm) and the other two at elevated (700 ppm) CO_2 concentrations. The injection of CO_2 to the TGG was structurally fixed, and therefore the same TGGs were set at elevated CO_2 concentration the three years of experiments. Each TGG has a modular design; temperature varied from ambient (following the external temperature diurnal cycle; module 1) to ambient $+4$ °C (adding 4 °C always to the external temperature; module 3). This was also structurally fixed. Finally, half of the plants growing inside each module were full irrigated and half were exposed to cyclic drought. The sub-sections to irrigate fully or partially the plants were also structurally fixed and the red and white Tempranillo plants positions within the modules of the TGGs were not assigned randomly to blocks. In the TGGs, CO_2 concentration and temperature were controlled and monitored, and relative humidity and radiation were monitored by an informatics system. Therefore, the treatments applied to the two grapevine varieties studied (red and white Tempranillo) were a combination of two CO_2 levels (ambient ca. 400 ppm, and elevated ca. 700 ppm), two temperature regimes (ambient and ambient $+4$ °C) and two water availability conditions (full irrigation and cyclic drought). Water treatments were applied and substrate water content recorded according to Kizildeniz et al. (2015).

2.4. Water treatments

Soil moisture sensors (EC-5 Soil Moisture Sensors, Decagon Devices Inc., Pullman, WA, USA) were placed into the pots (3 and 7 humidity sensors per treatment in full irrigated and cyclic drought treatments, respectively) during the last transplant (at fruit set). Substrate water content was controlled until maturity. Plants under full irrigation were maintained at ca. 80% of the substrate field capacity (sensor value between 30 and 40%, ($m^3 H_2O m^{-3}$ substrate) $\times$ 100; equivalent to 300–400 g $H_2O L^{-1}$ substrate). Cyclic drought consisted in withholding irrigation until plants showed visual signs of water deficit such as tendrils and leaves downwards (sensor value between 10 and 0%, ($m^3 H_2O m^{-3}$ substrate) $\times$ 100; equivalent to 0–100 g $H_2O L^{-1}$ substrate). When cyclic drought plants had symptoms, they were irrigated with the same amount of nutrients that received plants under full irrigation in that cycle.

2.5. Measurements and analyses

Gas exchange measurements were made on young, fully expanded leaves at the same physiological stage (from 8th to 10th node from the top) inside each TGG at the respective growth conditions of CO_2 (i.e., plants grown at current CO_2 were measured at 400 ppm CO_2, whereas those grown at elevated CO_2 were measured at 700 ppm), temperature and RH. Stomatal conductance (g_S) was performed using a portable photosynthesis system (LCi-SD with the PLUS5 compact light unit, ADC BioScientific, England) and it was measured in early morning under 1200 μmol $m^{-2} s^{-1}$ white LED light. Stomatal conductance was used as a reference parameter of the plant water stress at which plants were exposed (Medrano et al., 2002).

Plant leaf area was estimated using a model (Costanza et al., 2004), adapted for cv. Tempranillo. The model relates actual leaf area, which was measured with a leaf area meter (LI-300 model; Li-Cor Biosciences, Lincoln, USA) (y), and shoot length (x) (y = 15.5x + 24.8, R^2 = 0.93). Regression model was built over 40 shoots sampled throughout the growing season from extra plants.

Complete bunch of berries was collected at maturity (defined at 21–23 °Brix for cv. Tempranillo). Berry and rachis fresh weight (FW) were measured, and number of berries per bunch and seeds per berry were counted. Berry diameter was measured with a caliper. Relative skin mass was calculated as (skin FW/berry FW) $\times$ 100.

Leaves, petioles, shoots and roots were sampled at maturity, and then oven-dried at 80 °C for 48 h in order to obtain DW (or dry mass (DM)). Total vegetative mass was calculated as the sum of all these plant parts.

The design of the experiment was strip-strip-strip plot, with four factors (CO_2, temperature, water availability, and variety). The experiment was carried out three years (2013, 2014 and 2015; three biological replicates). Data were first tested using a four-way ANOVA (four factors: (i) CO_2 concentration (ii) temperature, (iii) water availability, and (iv) variety; and two levels, (i) 700 ppm CO_2 vs. 400 ppm, (ii) ambient temperature vs. temperature $+4$ °C, (iii) full irrigation vs. cyclic drought, and (iv) white vs. red Tempranillo) in order to determine the effects of the treatments and their possible interactions. Differences among groups were tested first with the Fisher's Least Significant Differences (LSD) post-hoc test and then with the Tukey test, the latter being quite conservative. These tests are useful when main effects or interactions are significant. Results were considered statistically significant at $p < 0.05$. Data are presented as means $\pm$ standard error (SE). Both absolute values and values relative to control (red Tempranillo, full irrigated, grown at current temperature and CO_2; set to 1) were used. This normalization was year based, i.e., control values of each year were set to 1 and all other treatment combinations were expressed relative to that year control. This eliminated year-to-year variation. The use of values relative to control was useful when variability among years was so high that blurred the effects of the applied treatments. All these statistical analyses were carried out with XLStat 7.5.2 Pro® statistical software (Addinsoft, Paris, France).

3. Results

3.1. Foreseen climate change and substrate water status at maturity in V. vinifera cv. red and white Tempranillo

Substrate water content is a useful parameter to monitor water use behavior of the two Tempranillo cultivars, red and white, and their

Table 3
Substrate water content (g $H_2O\,L^{-1}$ substrate) in red and white Tempranillo grown under different CO_2 concentrations (ambient (A_{CO2}) or 700 ppm CO_2 (E_{CO2})), temperature regimes (ambient (T) or ambient $+4\,°C$ (T + 4 °C)) and water availability (full irrigation (FI) or cyclic drought (CD)). Data were plotted as minimum (Min.) and maximum (Max.) mean (n = 3–7) during 3 years (2013, 2014 and 2015).

Years Treatments			period	2013 Min.–Max.	2014 Min.–Max.	2015 Min.–Max.
Red	**FI**	**T**	A_{CO2}	240–360	270–460	310–370
			E_{CO2}	290–430	260–440	300–380
		T + 4 °C	A_{CO2}	170–350	220–470	340–370
			E_{CO2}	180–360	120–340	290–370
	CD	**T**	A_{CO2}	0–310	20–300	10–300
			E_{CO2}	0–370	60–330	40–340
		T + 4 °C	A_{CO2}	0–340	20–320	10–330
			E_{CO2}	0–350	30–290	10–330
White	**FI**	**T**	A_{CO2}	290–380	220–430	320–380
			E_{CO2}	300–420	220–380	270–300
		T + 4 °C	A_{CO2}	250–390	210–420	260–320
			E_{CO2}	320–410	210–430	310–350
	CD	**T**	A_{CO2}	0–340	130–350	90–300
			E_{CO2}	10–390	80–300	80–280
		T + 4 °C	A_{CO2}	0–370	50–310	10–300
			E_{CO2}	0–390	30–280	50–340

response to the different stress factors. Cyclic drought resulted in half of the substrate water content, with respect to the full-irrigated control, mean values during the experiments were ca. 277–348 and 157–203 g $H_2O\,L^{-1}$ substrate in control and droughted plants respectively (Table 3). Just before re-irrigation, the lowest values of plants facing cyclic drought (Table 3) were below wilting point (Table 2). Elevated CO_2 and temperature did not affect substrate water content, averaged values were in the range 168–341 (elevated temperature), 157–348 (current temperature), 168–348 (elevated CO_2) and 157–334 (current CO_2) g $H_2O\,L^{-1}$ substrate (Table 3). All these values were between the value of permanent wilting point and that of field capacity (Table 2) for this substrate. Despite the abovementioned lowest values found in droughted plants, these values pointed to good water availability for the growth of the grapevine plants.

3.2. Foreseen climate change and stomatal conductance of V. vinifera cv. red and white Tempranillo

Stomatal conductance was used as a reference parameter of the extent of water stress that plants faced in the different treatments. Both red and white Tempranillo plants exposed to simulated climate change conditions (elevated CO_2 and temperature, and water deficit) had lower

stomatal conductance (g_S) than those grown under the current conditions (ambient CO_2 and temperature, and full irrigation) (Fig. 1). Statistical differences were observed using both Fisher's and Tukey tests. Effects of elevated CO_2, elevated temperature and drought on g_S were additive, showing significant decreases in response to CO_2 ($P_{(CO2)} = 0.009$), temperature ($P_{(Temp)} < 0.0001$) and water availability ($P_{(WA)} < 0.0001$) (Fig. 1). The ANOVA analysis also revealed two, three and four level significant interactions in the response of g_S to the simulated climate change (Fig. 1).

3.3. Foreseen climate change and plant growth at maturity of V. vinifera cv. red and white Tempranillo

Climate change conditions impacted growth in both red and white Tempranillo. Within the factors investigated, grapevine vegetative growth was highly influenced by water deficit. Water deficit decreased grapevine vegetative growth, reflected in significant decreases in both total vegetative mass (Fig. 2) and leaf area (Fig. 3) ($P_{(WA)} < 0.0001$ in both). This was observed both when absolute values and when values relative to control were considered (throughout this report, control is referred to red Tempranillo grown under full irrigation, ambient temperature and current CO_2; Figs. 2 and 3). Elevated CO_2 stimulated total vegetative mass ($P_{(CO2)} = 0.002$; Fig. 2), whereas had no influence in leaf area (Fig. 3). Contrary to this, no effects were observed in response to high temperature (Figs. 2 and 3). Data also revealed a significant higher ($P_{(Variety)} = 0.030$) total vegetative mass in red than in white Tempranillo (Fig. 2). The interaction between variety and high temperature ($P_{(Variety \times Temp)} = 0.030$; Fig. 2) evidenced that total vegetative mass of the two Tempranillo varieties under investigation did not respond similarly to temperature (see below).

In order to understand the behavior of individual and interacting factors in the whole vegetative growth, the DW of the different grapevine vegetative organs was studied (Table 4). Due to the variability among years, changes observed were better reflected in values relative to control (lower part of Table 4) than in absolute values (uppermost part of Table 4). Climate change conditions decreased leaf, petiole and shoot DW when compared to the current conditions in red Tempranillo, whereas only decreased petiole and shoot DW in the white one (Table 4). Both, Fisher's and Tukey tests gave significant differences. Drastic growth decreases due to water scarcity occurred in all organs ($P_{(WA)} < 0.0001$; Table 4), but this effect was more severe in shoots, petioles, and leaves than in roots (Table 4). These data indicate that water deficit impacted more in the above- than in the below-growth. On the contrary, elevated CO_2, when compared to current CO_2, increased leaf ($P_{(CO2)} = 0.007$), petiole ($P_{(CO2)} = 0.036$), shoot ($P_{(CO2)} = 0.011$)
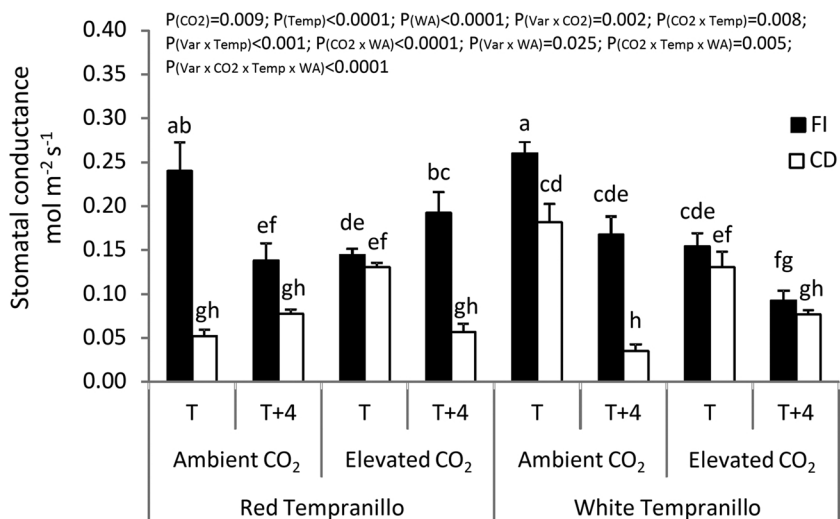
Fig. 1. Stomatal conductance recorded during a cycle of drought from fruit-bearing cuttings of red and white Tempranillo grown under two different CO_2 levels: elevated CO_2 (700 ppm) or ambient CO_2 (400 ppm), temperature regimes: high (T + 4 °C) or ambient (T) and irrigation treatments: full irrigation (FI) or water deficit (cyclic drought, CD). Values represent means (n = 4-6) ± SE. Within each parameter, means followed by different letters are significantly different ($P < 0.05$) based on Fisher's LSD test. When significant, P values of the main factors and interactions are shown.
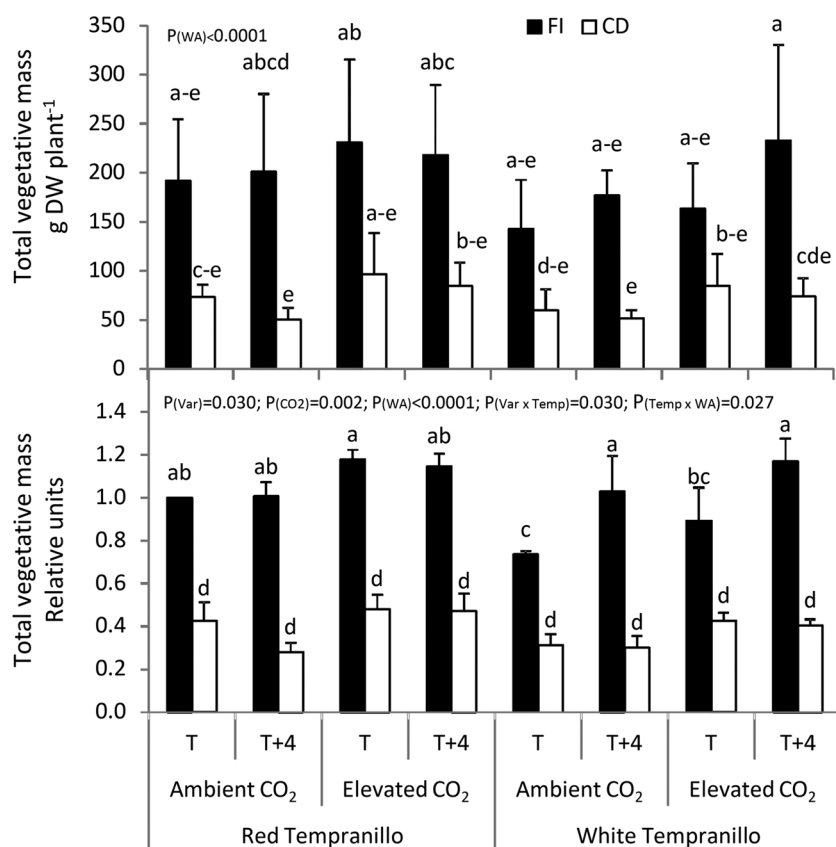
Fig. 2. Total vegetative mass recorded at harvest from fruit-bearing cuttings of red and white Tempranillo grown under two different CO_2 levels: elevated CO_2 (700 ppm) or ambient CO_2 (400 ppm), temperature regimes: high (T + 4 °C) or ambient (T) and irrigation treatments: full irrigation (FI) or water deficit (cyclic drought, CD). Values represent means (n = 3) ± SE. Within each parameter, means followed by different letters are significantly different (*P* < 0.05) based on Fisher's LSD test. When significant, *P* values of the main factors and interactions are shown. Uppermost plot, absolute values; lower plot, relative values to control set to 1 (see text).
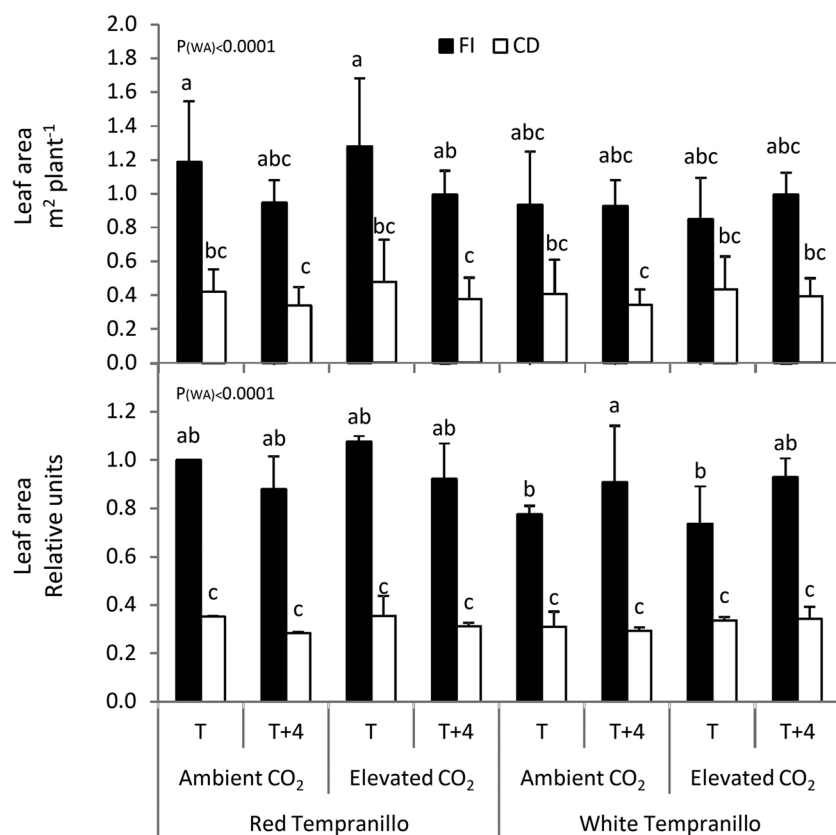
Fig. 3. Leaf area recorded at harvest from fruit-bearing cuttings of red and white Tempranillo grown under two different CO_2 levels: elevated CO_2 (700 ppm) or ambient CO_2 (400 ppm), temperature regimes: high (T + 4 °C) or ambient (T) and irrigation treatments: full irrigation (FI) or water deficit (cyclic drought, CD). Values represent means (n = 3) ± SE. Within each parameter, means followed by different letters are significantly different (*P* < 0.05) based on Fisher's LSD test. When significant, *P* values of the main factors and interactions are shown. Uppermost plot, absolute values; lower plot, relative values to control set to 1 (see text).

Table 4

Berry diameter, relative skin mass, number of seeds, leaf dry weight (DW), petioles DW, shoot DW and root DW in red and white Tempranillo grown under different CO_2 concentrations (ambient (A_{CO2}) or 700 ppm CO_2 (E_{CO2})), temperature regimes (ambient (T) or ambient + 4 °C (T + 4 °C)) and water availability (full irrigation (FI) or cyclic drought (CD)). Values represent means (n = 3) ± SE. Within each parameter, means followed by different letters are significantly different (P < 0.05) based on Fisher's LSD test. When significant, P values of the main factors and interactions are shown. Uppermost table, absolute values; lower table, relative values to control set to 1 (see text).

				Berry diameter	Relative skin mass	N° seeds	Leaf DW	Petiole DW	Shoot DW	Root DW
				mm	%FW		g DW plant^{-1}			
Red	A_{CO2}	T	FI	11.8 bc	29.5 a-d	1.37 ab	75.4 abc	9.7 abc	69.5 abc	35.6 a-e
			CD	11.2 c	24.3 cde	1.49 ab	24.4 c	2.9 bc	18.3 bc	24.8 cde
		T + 4	FI	12.7 abc	32.8 abc	1.22 ab	75.2 abc	8.8 abc	64.5 abc	49.9 abc
			CD	12.9 abc	22.7 de	1.87 a	18.8 c	2.3 c	13.2 c	15.2 e
	E_{CO2}	T	FI	13.5 abc	25.1 b-e	1.28 ab	87.5 ab	10.9 ab	79.7 a	53.6 ab
			CD	12.9 abc	24.1 cde	1.11 b	35.7 bc	4.0 abc	25.4 abc	29.2 b-e
		T + 4	FI	12.5 abc	26.0 a-e	1.65 ab	79.1 abc	8.6 abc	69.6 abc	56.2 a
			CD	13.4 abc	25.5 b-e	1.40 ab	25.4 bc	2.9 bc	20.5 bc	34.0 a-e
White	A_{CO2}	T	FI	13.9 ab	24.0 cde	1.47 ab	54.6 abc	7.4 abc	47.5 abc	32.1 a-e
			CD	12.7 abc	20.0 e	1.48 ab	21.0 c	2.7 bc	16.2 bc	16.7 e
		T + 4	FI	13.1 abc	26.2 a-e	1.32 ab	69.4 abc	8.6 abc	55.9 abc	39.6 a-e
			CD	12.6 abc	34.4 a	1.28 ab	18.5 c	2.2 c	11.8 c	14.5 e
	E_{CO2}	T	FI	14.4 a	25.7 a-e	1.34 ab	71.2 abc	7.4 abc	47.3 abc	35.6 a-e
			CD	12.6 abc	32.5 abc	1.23 ab	32.4 bc	3.7 abc	22.8 abc	23.7 de
		T + 4	FI	13.3 abc	33.5 ab	1.39 ab	101.0 a	10.5 ab	73.3 ab	46.7 a-d
			CD	13.6 ab	24.5 cde	1.45 ab	28.6 bc	3.3 abc	18.9 bc	23.3 de
Cultivar(Var)				0.122	0.382	0.651	0.778	0.691	0.416	0.074
CO_2				0.125	0.800	0.497	0.242	0.556	0.463	**0.048**
Temperature (Temp)				0.710	0.107	0.402	0.874	0.892	0.990	0.439
Water availability (WA)				0.331	0.237	0.764	**< 0.0001**	**0.0001**	**0.0001**	**< 0.0001**
Var*CO2				0.544	0.108	0.678	0.682	0.915	0.990	0.561
CO2*Temp				0.510	0.196	0.279	0.995	0.997	0.870	0.818
Var*Temp				0.352	0.324	0.313	0.475	0.459	0.534	0.914
Temp*WA				0.119	0.522	0.534	0.507	0.758	0.646	0.241
CO2*WA				0.741	0.549	0.198	0.777	0.938	0.956	0.908
Var* WA				0.325	0.137	0.649	0.845	0.720	0.530	0.653
Var*CO2*Temp				0.237	0.165	0.843	0.763	0.726	0.778	0.941
Var*CO2* WA				0.891	0.110	0.213	0.745	0.962	0.975	0.861
CO2*Temp* WA				0.520	0.076	0.672	0.885	0.932	0.883	0.501
Var*Temp* WA				0.826	0.950	0.736	0.620	0.537	0.561	0.992
Var*CO2*Temp* WA				0.826	**0.011**	0.386	0.820	0.802	0.783	0.444

				Berry diameter	Relative skin mass	N° seeds	Leaf DW	Petiole DW	Shoot DW	Root DW
				Relative units						
Red	A_{CO2}	T	FI	1.00 bc	1.00 a-d	1.00 ab	1.00 ab	1.00 a	1.00 ab	1.00 b-e
			CD	0.95 c	0.82 bcd	1.10 ab	0.35 d	0.32 d	0.27 e	0.69 ef
		T + 4	FI	1.08 abc	1.12 ab	0.89 b	1.02 ab	0.94 ab	0.91 bc	1.35 abc
			CD	1.10 abc	0.79 cd	1.36 a	0.26 d	0.27 d	0.19 e	0.41 f
	E_{CO2}	T	FI	1.14 abc	0.86 a-d	0.96 b	1.18 a	1.10 a	1.14 a	1.48 ab
			CD	1.10 abc	0.84 a-d	0.83 b	0.42 cd	0.38 d	0.31 e	0.79 def
		T + 4	FI	1.06 abc	0.89 a-d	1.20 ab	1.13 a	0.96 ab	1.02 ab	1.54 a
			CD	1.14 abc	0.90 a-d	1.03 ab	0.36 d	0.34 d	0.28 e	0.92 c-f
White	A_{CO2}	T	FI	1.18 ab	0.83 a-d	1.06 ab	0.72 bc	0.77 c	0.68 d	0.90 c-f
			CD	1.07 abc	0.69 d	1.10 ab	0.27 d	0.26 d	0.21 e	0.46 f
		T + 4	FI	1.11 abc	0.90 a-d	0.96 b	1.11 a	0.99 a	0.90 bc	1.12 a-e
			CD	1.06 abc	1.15 a	0.95 b	0.26 d	0.26 d	0.17 e	0.41 f
	E_{CO2}	T	FI	1.22 a	0.89 a-d	0.99 b	1.05 a	0.80 bc	0.73 cd	0.99 b-e
			CD	1.06 abc	1.10 abc	0.92 b	0.41 cd	0.36 d	0.29 e	0.66 ef
		T + 4	FI	1.13 abc	1.14 ab	1.02 ab	1.30 a	1.09 a	1.04 ab	1.28 a-d
			CD	1.16 abc	0.85 a-d	1.07 ab	0.42 cd	0.36 d	0.26 e	0.65 ef
Cultivar (Var)				0.168	0.495	0.554	0.669	0.126	**0.005**	**0.025**
CO_2				0.151	0.712	0.432	**0.007**	**0.036**	**0.011**	**0.011**
Temperature (Temp)				0.709	0.128	0.135	0.317	0.399	0.555	0.342
Water availability (WA)				0.356	0.270	0.586	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
Var*CO2				0.546	0.146	0.632	0.428	0.773	0.938	0.430
CO2*Temp				0.543	0.231	0.169	0.717	0.961	0.601	0.759
Var*Temp				0.366	0.429	0.211	0.072	**0.005**	**0.007**	0.785
Temp*WA				0.145	0.586	0.454	0.097	0.139	0.077	0.132
CO2*WA				0.736	0.501	0.082	0.439	0.799	0.604	0.859
Var* WA				0.339	0.227	0.611	0.789	0.317	**0.037**	0.562
Var*CO2*Temp				0.255	0.252	0.787	0.840	0.628	0.822	0.993
Var*CO2* WA				0.894	0.130	0.125	0.816	0.800	0.713	0.729
CO2*Temp* WA				0.534	0.126	0.628	0.568	0.909	0.986	0.364
Var*Temp* WA				0.854	0.977	0.604	0.270	**0.022**	**0.015**	0.990
Var*CO2*Temp* WA				0.841	**0.022**	0.268	0.882	0.571	0.543	0.335

and root ($P_{(CO2)}$ = 0.011) DW, whereas high temperature had no much influence (Table 4). Thus, there was a significant interaction in petiole and shoot DW ($P_{(Variety \times Temp \times WA)}$ = 0.022 and 0.015, respectively; Table 4). This corresponded to a positive effect of temperature only in well-watered plants of the white Tempranillo cultivar. White Tempranillo grew less than the red one (Table 4), with significant lower values in shoot ($P_{(Variety)}$ = 0.005) and root ($P_{(Variety)}$ = 0.025) DW.

Analysis of the interaction between water deficit and varieties revealed a significantly higher reduction in shoot growth due to the water deficit in red than in white Tempranillo ($P_{(Variety \times WA)}$ = 0.037; Table 4). Given that Var*WA interaction is included in the Var*-Temp*WA interaction, which was significant for shoot DW, shoot DW reduction produced by water stress was only observed at actual temperature, not at temperature +4 °C (Table 4).

Interestingly in well-watered plants, elevated temperature had a positive influence on the growth of white Tempranillo plants, whereas its influence was not so clear in the red one (Table 4). Temperature increased the DW of petioles and shoots in white Tempranillo, while decreases were observed in the red variety (Table 4; $P_{(Variety \times Temp)}$ = 0.005 and 0.007, respectively). The different behavior of the two varieties, and especially the increased growth of some particular organs of the white one, could be a sign of the existence of a different physiological response to temperature.

3.4. Foreseen climate change and reproductive growth at maturity of V. vinifera cv. red and white Tempranillo

Reproductive growth was unaffected by the simulated climate change scenario, both in red and white Tempranillo. Parameters analyzed were number of berries per bunch, number of seeds per berry, berry diameter, berry and rachis FW and relative skin mass. Neither Fisher's nor Tukey tests gave differences. In red Tempranillo, there was a tendency to produce a few less berries per bunch under drought but differences did not reach statistical significance (Fig. 4). Only small

differences could be observed. Rachis FW was lower in droughted plants than in the full irrigated ones ($P_{(WA)}$ = 0.035; Fig. 5), whereas berry FW was higher in plants grown under elevated CO_2 than in those grown at current CO_2 concentration ($P_{(CO2)}$ = 0.037; Fig. 6). Also, ANOVA revealed a four level interaction in relative skin mass (Table 4; $P_{(Variety \times CO2 \times Temp \times WA)}$ = 0.011 and 0.022, when absolute or relative values were considered respectively).

4. Discussion

Data recently reported indicates that red Tempranillo consumes more water than the white one (Kizildeniz et al., 2015). Causes for such higher water consumption in the red cultivar may include a higher root density and/or a larger leaf area at plant level. Not all the abovementioned factors contribute to the observed varietal differences. Plant root and shoot DW was higher in red than in white Tempranillo, but plant leaf area was similar in both cultivars. Also, our preliminary, unpublished results indicate that the stomatal density is lower in white Tempranillo when compared to the red one. It is very likely that the natural mutation (with genetic material loss; Carbonell-Bejerano et al., 2017) that originated the white variety from red Tempranillo had affected loci other than those related to berry color. Regarding water consumption and leaf area, drought cycles length was reported to be shorter and symptoms of water deficit appeared with lower water content in the growth substrate at the end than at the beginning of the experiments in both red and white Tempranillo (Kizildeniz et al., 2015), which at least in part might be related to the leaf area growth and development of the Tempranillo plants.

Water scarcity was found to be the most detrimental factor related to climate change, causing a drastic reduction in plant vegetative growth (including shoots and roots), in line with previous reports (Schultz and Matthew, 1988). From our results, it is clear that the highest effect was induced by water reduction, which, given the experimental setup, it was also the most extreme treatment: temperature
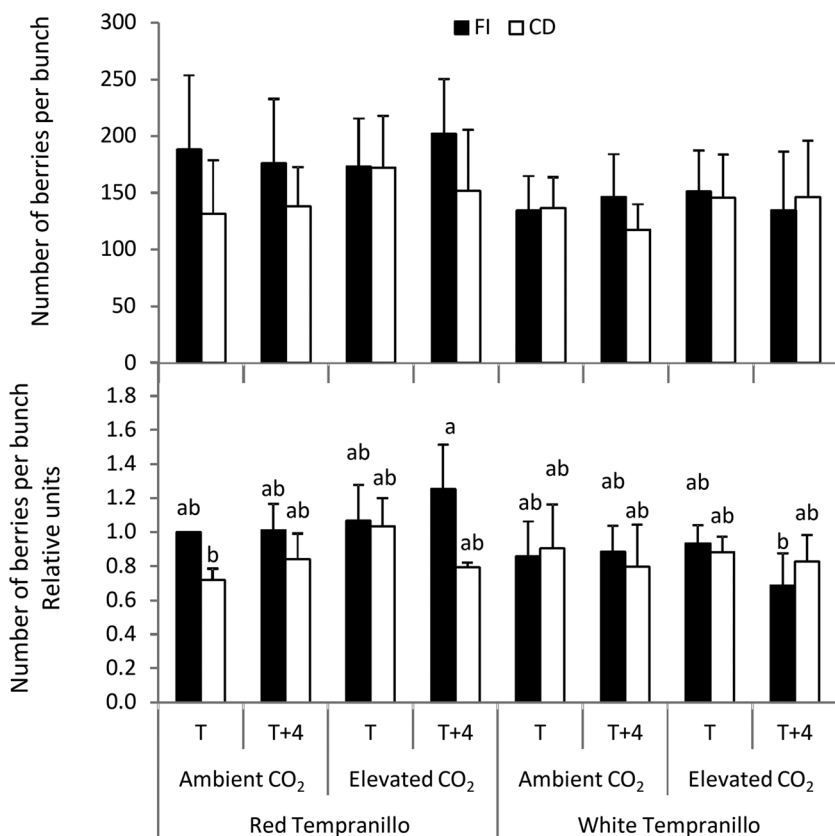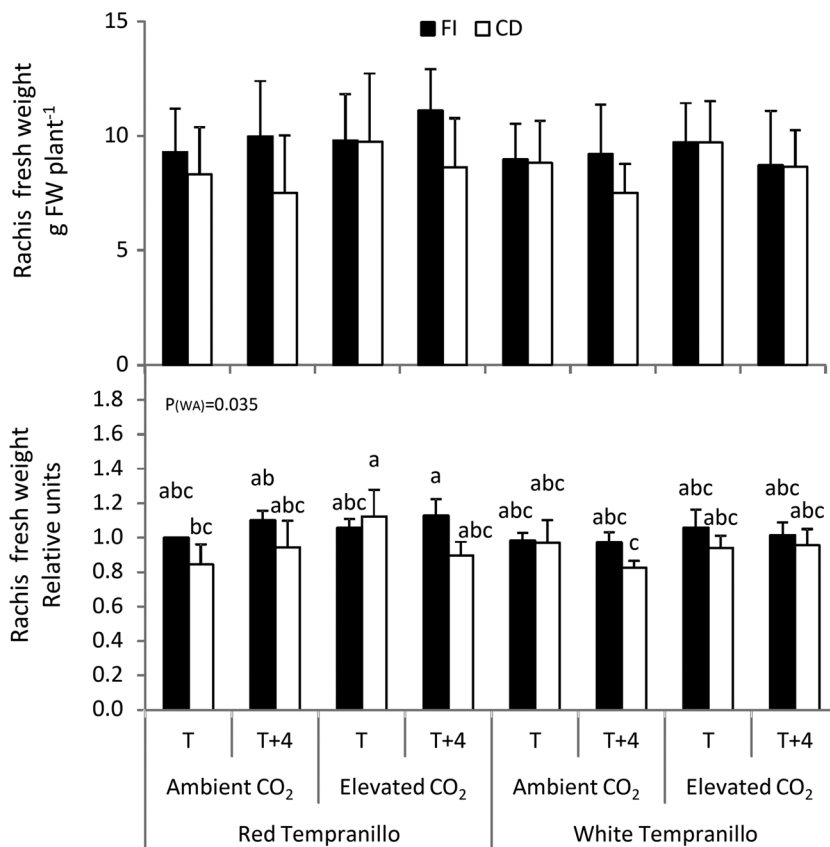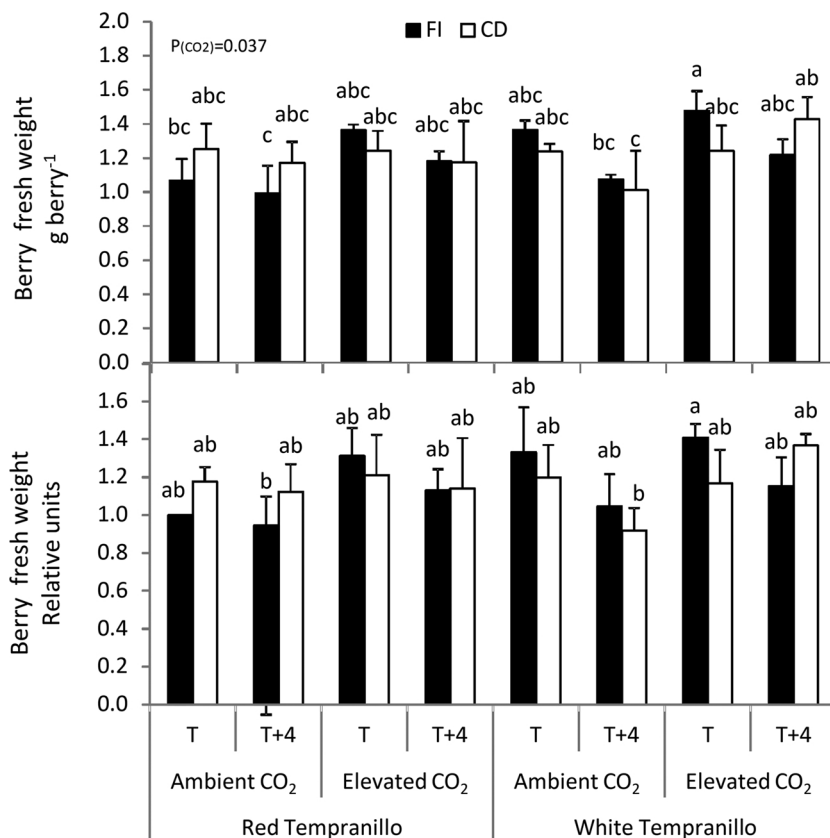

Fig. 4. Number of berries per bunch recorded at harvest from fruit-bearing cuttings of red and white Tempranillo grown under two different CO_2 levels: elevated CO_2 (700 ppm) or ambient CO_2 (400 ppm), temperature regimes: high (T + 4 °C) or ambient (T) and irrigation treatments: full irrigation (FI) or water deficit (cyclic drought, CD). Values represent means (n = 3) ± SE. Within each parameter, means followed by different letters are significantly different (P < 0.05) based on Fisher's LSD test. When significant, P values of the main factors and interactions are shown. Uppermost plot, absolute values; lower plot, relative values to control set to 1 (see text). No letters in the uppermost plot means that there were no significant differences.

Fig. 5. Rachis fresh weight recorded at harvest from fruit-bearing cuttings of red and white Tempranillo grown under two different CO_2 levels: elevated CO_2 (700 ppm) or ambient CO_2 (400 ppm), temperature regimes: high (T + 4 °C) or ambient (T) and irrigation treatments: full irrigation (FI) or water deficit (cyclic drought, CD). Values represent means (n = 3) ± SE. Within each parameter, means followed by different letters are significantly different ($P < 0.05$) based on Fisher's LSD test. When significant, P values of the main factors and interactions are shown. Uppermost plot, absolute values; lower plot, relative values to control set to 1 (see text). No letters in the uppermost plot means that there were no significant differences.

Fig. 6. Berry fresh weight recorded at harvest from fruit-bearing cuttings of red and white Tempranillo grown under two different CO_2 levels: elevated CO_2 (700 ppm) or ambient CO_2 (400 ppm), temperature regimes: high (T + 4 °C) or ambient (T) and irrigation treatments: full irrigation (FI) or water deficit (cyclic drought, CD). Values represent means (n = 3) ± SE. Within each parameter, means followed by different letters are significantly different ($P < 0.05$) based on Fisher's LSD test. When significant, P values of the main factors and interactions are shown. Uppermost plot, absolute values; lower plot, relative values to control set to 1 (see text).

was increased in 4 °C, a situation the plant confronts usually; CO_2 concentration was increased in 75%, not a severe increase for the plant capacities; but for watering, one of the levels of the treatment was unreal (although unavoidable in potted plants): full capacity is never present in Mediterranean conditions, plants, even with support irrigation, are between drought and severe drought most of their life cycle. Moreover, substrate characteristics, which were far from the typical clay-calcareous soils quite common in vineyards, could also affect the water stress response. These may be the reasons why water availability had the highest effect.

Low vegetative growth was associated to low substrate water status in cyclic drought-grown plants, being this result consistent the three years of experiments. Also, the water stress imposed was reflected in lower stomatal conductances in droughted plants when compared to the full irrigated ones. Root growth was less susceptible to water deficit than the abovegrown organs: leaves, petioles and shoots, as was also indicated previously by Williams and Matthews (1990) and Kizildeniz et al. (2015). There was no interaction between water availability and either CO_2 or temperature with respect to vegetative growth. It should be also remarked that growth of red Tempranillo was more impacted by drought than that of the white one.

Effects of future climate change on crop productions are likely to vary widely among crops and regions (Moriondo et al., 2010). In Southern Europe, because of water shortage and extreme weather events (heat and drought), agriculture is expected to face large yield losses (Bindi and Olesen, 2011; Ferrise et al., 2011; Moriondo et al., 2011; Ruiz-Ramos et al., 2011). Negative effects of water stress on reproductive growth had been widely reported (McCarthy, 1997; Petrie et al., 2004; Santesteban et al., 2011). However, it can be concluded from the literature that the impact of water stress on grapevines is more severe in vegetative than in reproductive growth (Korkutal et al., 2011; Williams and Matthews, 1990). Our data indicate no changes in number of berries per bunch, berry diameter and FW, relative skin mass, and number of seeds per berry in response to drought. At the beginning of the berry developmental stage, berry growth is very sensitive to water stress and the effects of drought cannot be reversed by supplemental irrigation during the following stages. Nevertheless, applying climate change related factors after fruit set, including drought, has minor effects on berry size, as has been previously reported by Mekni (2014) and Kizildeniz et al. (2015) using grapevine fruit bearing cuttings.

Changes in thermal conditions predominantly regulate grapevine phenological cycle, including critical periods such as bud break, flowering, berry growth, veraison, maturation, leaf fall, and dormancy (Iland et al., 2011). Warming temperatures are related to a shorter period between bud burst and harvest, inducing earlier flowering, veraison, ripening and harvest (Duchêne and Schneider, 2005; Keller, 2010; Martínez-Lüscher et al., 2016). Over a 50-year period, grape harvest now almost occurs one month earlier (Seguin and de Cortazar, 2005). During the vegetative cycle, grapevine plants should choose between opening stomata with the aim of maintaining as low as possible leaf temperature and as high as possible photosynthesis, or closing them preventing unwanted water losses and excessive dehydration (Campitelli and Stinchcombe, 2013; Chaves et al., 2016; Jones, 2014; Nicotra et al., 2008; Peppe et al., 2011). Despite the closure of stomata observed in this work induced by the elevated temperature, our data indicate no effects on vegetative or reproductive growth increasing the temperature 4 °C.

When nutrients and water are available to plants, elevated CO_2 accelerates grapevine growth (Mullins et al., 1992) and yield (with no negative impacts on grape and wine quality; Bindi et al., 2001), but to a different extent among cultivars (Kizildeniz et al., 2015). Elevated CO_2 stimulated more vegetative (total vegetative mass) than reproductive (berry FW) growth. Elevated CO_2 increased leaf, petiole, shoot and root DW, and there were no interactions with temperature or water availability. The beneficial effects of elevated CO_2 on growth are likely related to the higher rates of photosynthesis reported in grapevines grown

under such high CO_2 concentrations (Bindi et al., 1996, 2001; Da Silva et al., 2017; Salazar-Parra et al., 2012, 2015; Schultz, 2000). Stomatal conductance and transpiration rates (Flexas et al., 2014; Leakey et al., 2009; Salazar-Parra et al., 2012, 2015) as well as stomatal density (Moutinho-Pereira et al., 2009; Rogiers et al., 2011) are reduced by elevated CO_2 exposure. The increases in photosynthesis and decreases in transpiration lead to an improved water use efficiency (Da Silva et al., 2017; Wullschleger et al., 2002). In contrast to previous reports (Chaves and Pereira, 1992; Kizildeniz et al., 2015; Williams and Matthews, 1990), elevated CO_2 did not compensate for the negative effects of water stress on vegetative growth under our experimental conditions.

5. Conclusions

Climate change scenario impacted vegetative growth in red and white Tempranillo. Plant vegetative growth decreased especially with water deficit treatments. The inhibitory effect of water deficit was related to low substrate water status and low stomatal conductance. Elevated CO_2 stimulated vegetative growth, in particular total vegetative mass (leaf area was not affected). In white and red Tempranillo, the largest increases when plants were grown under elevated CO_2 were observed in leaf and root growth, respectively. In contrast to previous reports of grapevine, elevated CO_2 did not compensate the negative effects of water stress. An increase of the mean temperature 4 °C had no consequences on vegetative growth. Yield and yield-related traits were unaffected by the climate change scenario.

Acknowledgements

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